Combined Report

Physiological and Biochemical Neuroprotection in Cetaceans: Are Some Marine Mammal Species Safeguarded from Emboli Formation and Barotrauma?

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Predicting Natural Neuroprotection in Marine Mammals: Environmental and Biological Factors Affecting Vulnerability to Acoustically Mediated Tissue Trauma in Marine Species

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LONG-TERM GOALS

The primary goal of these studies is to investigate the relative vulnerability of marine mammals to acoustically mediated trauma from emboli formation. By evaluating key environmental, behavioral and physiological factors involved in the movement of gases at the whole animal and tissue levels we intend to identify factors contributing to lipid and nitrogen gas mobilization, and concomitant tissue damage at depth. The results of this project will enable the development of environmentally sensitive schedules for oceanic acoustic activities by identifying those species most susceptible to tissue injury.

OBJECTIVES

To accomplish these goals we are focusing on three key questions:

1. **Environmental:** ***Does elevated environmental temperature compromise the dive response that safeguards marine mammals from decompression illness?*** This is being tested by measuring cardiovascular and metabolic parameters of trained bottlenose dolphins during sedentary and active periods while diving in warm and cold water.

2. **Behavioral:** ***Do increased levels of neuroprotecting globins in the brain correspond to increased plasticity of the dive response during voluntary activity by marine mammals?*** Here we evaluate the physiological significance of elevated globin levels that we have discovered in the cerebral cortex of marine mammals. This is being tested by comparing behaviorally induced variability in the dive response (as manifested by changes in the level of bradycardia and peripheral circulation) in deep and shallow diving mammal species including bottlenose dolphins and beluga whales.

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14. ABSTRACT
The primary goal of these studies is to investigate the relative vulnerability of marine mammals to acoustically mediated trauma from emboli formation. By evaluating key environmental, behavioral and physiological factors involved in the movement of gases at the whole animal and tissue levels we intend to identify factors contributing to lipid and nitrogen gas mobilization, and concomitant tissue damage at depth. The results of this project will enable the development of environmentally sensitive schedules for oceanic acoustic activities by identifying those species most susceptible to tissue injury.

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3. **Physiological:** ***Does globin deposition and coincident neuroprotection of the cerebral cortex change with developmental stage in marine mammals?*** By evaluating globin deposition profiles from carcasses ranging in age from neonates to adults, we are investigating how age influences neuroprotective mechanisms in a wide variety of marine mammal species.

Together these studies will enable us to determine if some marine mammal species, such as the family of beaked whales, are more susceptible to non-auditory tissue damage as may occur in conjunction with navy and oil exploration sound operations. We will take into account several recent hypotheses regarding emboli formation, observed behavioral responses of marine mammals to low- and mid-frequency sound production, as well as the results of our studies to develop predictive models for susceptibility to decompression illness.

APPROACH

This study uses two approaches to determine the relative susceptibility of different marine mammal species to acoustically mediated trauma, 1) molecular and biochemical evaluation of neuroprotection at the tissue level, and 2) whole animal/physiological assessments to determine the impact of behavioral and environmental challenges to the dive response. Because stranded marine mammals often display behaviors associated with neural dysfunction (i.e. disorientation, poor localization and righting responses), and neural tissues are exceptionally vulnerable to decompression damage, we focus on the central nervous system and its relationship to the dive response.

Laboratory studies at the tissue level are assessing the presence and function of oxygen binding circulating (hemoglobin) and resident (cytoglobin and neuroglobin) globin proteins in the brain. Recently, a survey of shallow and deep diving species enabled us to determine the effects of routine dive capacity on the expression of these globins (Williams *et al.,* 2008). Our current studies build on this foundation to evaluate how these different globins affect the vulnerability of a variety of marine mammal species to hypoxia associated with decompression syndromes. Because the concentration of other globin proteins (i.e. myoglobin) changes with developmental stage in marine mammals, we are also examining how age influences globin deposition and coincident neuroprotection in the brain of immature and mature marine mammals. Ultimately, this will allow us to determine if specific segments of marine mammal populations are more susceptible than others to neural damage. Team members include specialists in morphology and pathology of marine mammals (M. Miller, CA Dept. Fish and Game; D.A. Pabst, Univ. North Carolina-Wilmington), globin chemists (D. Kliger and R. Goldbeck, UCSC), molecular biologists (M. Zavanelli, UCSC) and physiologists (T.M. Williams and D. Casper, UCSC).

The second component of this study examines the susceptibility of marine mammals to decompression illness at the whole animal/physiological level by monitoring behaviorally induced variability in the dive response. Because nitrogen transfer and decompression illness are linked to tissue perfusion, relaxation of the dive response in marine mammals has the potential to increase susceptibility to neural tissue damage either by preventing the removal of nitrogen or altering the perfused tissue pool available for nitrogen dispersal. The effects of two physiological mechanisms known to alter blood flow are being investigated, exercise and heat. In the first series of tests we are evaluating the effects of exercise intensity on changes in the dive response of bottlenose dolphins. Dolphins are trained to dive and exercise at varying depths. Variability in bradycardia and peripheral vasoconstriction are subsequently monitored as the animals perform sedentary to high intensity exercise tasks. Our most recent work provides a comparative dimension by conducting similar tests on a deep diving species, the beluga whale. A second set of tests uses this protocol is determining the effects of acute and chronic increases in environmental temperature on variability of the dive response in diving bottlenose dolphins.

Team members for this part of the program include physiologists (T.M. Williams, S. Noren, and L. Yeates from UCSC) and animal behaviorists (T. Kendall and B. Richter, UCSC; P. Berry, EPCOT; W. Hurley, GA Aquarium)

WORK COMPLETED

Tissue Globin Analyses. Our team has successfully developed two assays for brain globins, a spectrophotometric test that provides total globin concentration and an mRNA expression test for relative cytoglobin and neuroglobin levels. Previously, we have used these assays to detect the presence and concentration of globins in the cerebral cortex of 16 species of mammals. This includes five species of terrestrial mammal ranging in body mass from 0.1 kg to 100 kg, and 11 species of marine mammal ranging in mass from 30 to 300 kg. Among the marine species, we have examined both coastal and pelagic divers among the small cetaceans, pinnipeds and sea otters. All have demonstrated the presence of globins, although the concentration varies among the various species. We are currently refining our isolation techniques in order to quantify the level of globins as well as characterize the exact molecular structure of the globins. Furthermore, we have collected brain samples representing different ages from several marine mammal species including sea otters, pinnipeds and cetaceans. These are scheduled for analysis in January 2010.

Variation in Diving Bradycardia. The second component of this study examines variability in the dive response of cetaceans. A major challenge has been developing heart signal instrumentation that could withstand the rapid swimming movements of dolphins. This year we successfully tested and collected data using a new submersible electrocardiograph monitor by UFI (Morro Bay, CA). Eight dolphins and two beluga whales have been examined. Heart rate during surface and submerged resting periods were collected for both species. In addition, we completed a series of exercise tests for dolphins freely-diving to 3 m, 10 m and 20 m. Data analysis is ongoing to establish the heart rate signatures for each level of exercise intensity. These will be used as a template for conducting identical activity tests with the beluga whales. The results from the dolphin tests are scheduled for presentation at the upcoming Society for Integrative and Comparative Biology meetings (Seattle WA, January 2010). Predictive models of gas movement in the cardiovascular system, aerobic dive limits, and susceptibility to decompression illness based on our results are currently being developed for deep diving and fast swimming classes of marine mammals.

RESULTS

A major accomplishment during this year was completion of the first series of exercise tests for diving bottlenose dolphins. With the development of an ECG-accelerometer microprocessor we have, 1) correlated discrete changes in heart rate with propulsive stroking, 2) determined the effects of preferred and trained exercise on diving bradycardia, and 3) examined the variation in interbeat interval during diving bradycardia for low and high speed swimming (Fig. 1). Initial tests indicate considerable variation in the level of bradycardia maintained by submerged dolphins. Rather than an inflexible response diving bradycardia depended on activity state. The heart rate of adult bottlenose dolphins ranged from approximately 30 - 120 beats.min^{-1}. The highest and lowest heart rates exhibited by the

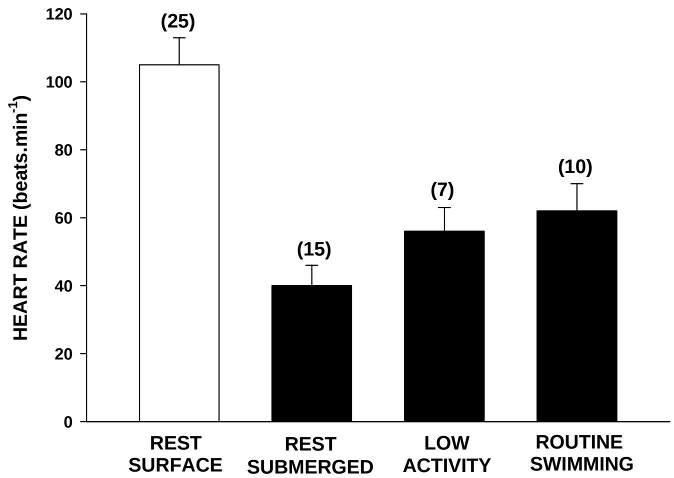

Figure 1. Variability in diving heart rate in the bottlenose dolphin. The white bar denotes the respiratory tachycardia exhibited by animals resting on the water surface. The black bars demonstrate the changes in heart rate that occur with increased exercise intensity during submergence. Bar height and lines denote mean + 1 SE. Numbers in parentheses are trial number.

dolphins occurred while stationing on the water surface and while stationing on a submerged target, respectively. Submerged activity resulted in considerable variability in the level of bradycardia maintained in the diving animals, progressively increasing from sedentary stationing to slowly swimming at preferred speeds to rapid maneuvers such as quick turns or ascents. Detailed tests with beluga whales and for dolphins exercising at specific loads are ongoing.

For the tissue portion of the project, we focused on tissue collection in order to conduct a comparative study on the effects of age on globin deposition. With the current collection of cerebral cortex samples from immature and mature sea otters, harbor porpoise, and harp, hooded and Weddell seals we are ready to conduct an analysis concerning the interrelationship between tissue globin level, dive capability, and animal age. Both molecular and biochemical analyses will occur simultaneously.

Lastly, with basic information regarding heart rate variability and tissue globin levels from our studies we have begun constructing a predictive model to determine species specific susceptibility to decompression illness. Major oxygen stores, skeletal muscle characteristics, globin deposition, diving capability, and cardiovascular variability are being incorporated into the model. Thus far, comparisons between terrestrial and marine mammal species have revealed marked differences for all of these factors. For example, Figure 2 demonstrates the variability in one factor, skeletal muscle characteristics of the major locomotory muscles for odontocetes and other mammals. Similar muscles exhibit markedly different profiles and correlate with observed performance levels.

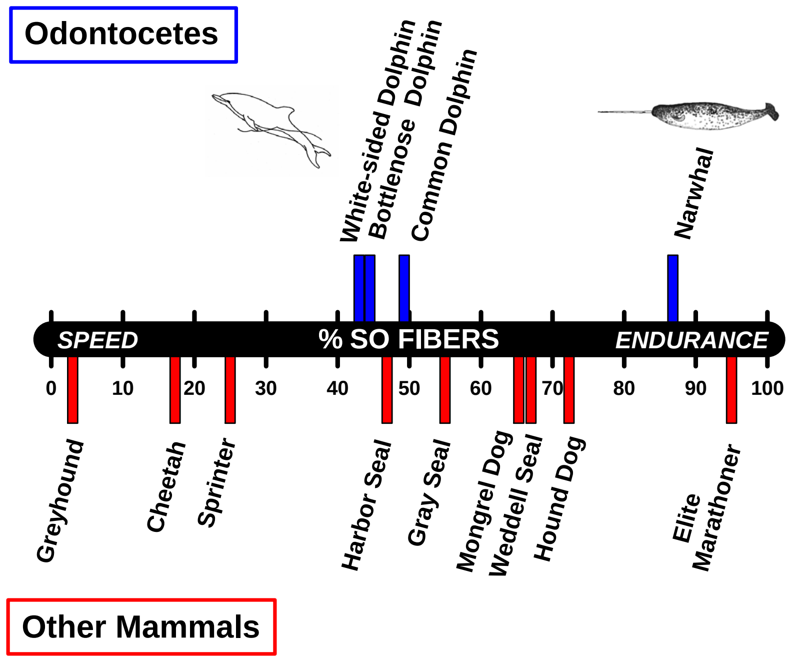

Figure 2. Variability in the percentage slow oxidative fibers in the major locomotory muscles of mammals. Values for the bottlenose dolphin are compared to those of similar muscles powering the flukes of other odontocetes (upper panel) as well as swimming and running skeletal muscles of pinnipeds and terrestrial mammals, respectively (lower panel). Fast swimming odontocetes maintain relatively mixed fiber types in the propulsive muscles while slower swimming, deeper diving species such as the narwhal show a larger proportion of slow twitch fibers. (From Williams et al. submitted.)

These preliminary results indicate that neuroprotection in diving marine mammals may be a species-specific balance between intrinsic and extrinsic factors. A suite of oxygen-binding globins appear to provide complimentary mechanisms for facilitating oxygen transfer into neural tissues as well as the potential for protection against reactive oxygen and nitrogen groups when marine mammals are submerged. A variable cardiovascular response when submerged enables the animals to meet the demands of exercise but raises a question regarding the movement of gases (oxygen, carbon dioxide and nitrogen) during diving. The vulnerability of essential tissues to injury during submergence will be addressed following further tests and the development of a model that incorporates the variability in tissue profiles and physiological responses observed in these studies.

IMPACT/APPLICATIONS

Our recent findings on variability in the cardiovascular response to diving and in tissue globin levels in the cerebral cortex provide:

1. A new perspective on neuroprotection. By examining a wide variety of mammalian species living in different habitats, we demonstrate how malleable the mammalian brain can be when placed under extreme chronic hypoxia, which occurs not only in air-breathing vertebrates who dive but also in response to various common medical conditions in humans and other species.

2. An assessment of the importance of globin proteins. Since neuroglobin and cytoglobin have been associated with neuronal survival following stroke and other ischemic insults with cardiovascular accidents, the results are relevant to many of the leading causes of mortality in the United States. Furthermore, although further research is needed, differences in resident neuroglobins may help to explain the relative susceptibility of deeper diving species to barotrauma following exposure to anthropogenic noise.

3. New techniques for clinical, ecological, behavioral and physiological studies. The instrumentation developed for monitoring cardiovascular changes in freely-diving marine mammals provides a new tool for assessing the response of wild mammals to anthropogenic disturbance. In addition, our study is developing new biochemical methods and animal models for the assessment of brain globins that should be of interest to a wide variety of comparative and medical neurophysiologists.

RELATED PROJECTS

None.

PUBLICATIONS AND PRESENTATIONS

Noren, S.R., Williams, T.M., Kendall, T., and Cuccurullo, V. (2009) Bradycardia Redefined: A Variable cardiovascular dive response in dolphins. **Integrative and Comparative Biology**, Seattle WA, January 2010 [submitted, refereed].

Williams, T.M., Zavanelli, M., Miller, M.A., Goldbeck, R.A., Morledge, M., Casper, D., Pabst, D.A., McLellan, W., Cantin, L.P., and Kliger, D.S. (2008) Running, swimming and diving modifies neuroprotecting globins in the mammalian brain. **Proceedings Royal Society of London** 275, 751-758 [published, refereed].

Williams, T.M., Noren, S.R., and Berry, P.S. (2009) “Bending” the rules: The role of cardiovascular exercise responses in protecting the brain of diving marine mammals. **Integrative and Comparative Biology**, Seattle WA, January 2010 [submitted, refereed].

Williams, T.M., Noren, S.R., and Glenn, M. (2009) Extreme Physiological Adaptations as Predictors of Climate-Change Sensitivity in the Narwhal, *Monodon Monoceros*. **Marine Mammal Science** [submitted, refereed].

HONORS/AWARDS/PRIZES

T.M. Williams (2009) Laurence Irving- Per Scholander Memorial Lecturer, University of Alaska, Fairbanks